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Abstract

The main goal of this project is to investigate whether polymorphisms of the CYP1B1 gene can be a risk factor for race-related prostate cancer. Two specific aims were proposed and are as follows: 1) To test the hypothesis that CYP1B1 gene is hyper-activated during malignant transformation of race-related prostate cells; and 2) To test the hypothesis that single nucleotide polymorphisms (SNPs) of the CYP1B1 gene have higher risk for race-related prostate cancer and correlate with hyper-activated CYP1B1 gene. We have obtained 77 benign prostatic hyperplasia (BPH) and 57 prostate cancer (PC) samples from African-American and Caucasian patients. The first hypothesis is under experimentation. To analyze whether SNPs of CYP1B1 gene are risk factors for race-related PC, two polymorphic sites at codons 119 and 432 have been evaluated. Based on the samples obtained, between races, the variant allele at codon 432 appears to be a risk factor among Blacks compared to Whites for both BPH ($P < 0.01$) and PC ($P = 0.06$). No difference was detected at codon 119. Within race, no differences were observed between BPH and PC at either SNP site. The continuation of SNP studies with additional samples to be collected and experimentation with aim #1 will be the focus in years 2 and 3.

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INTRODUCTION

Prostate cancer is the most frequently diagnosed malignancy and the second leading cause of death among men with cancer in the USA. When comparing races, the incidence and mortality rates of prostate cancer in African-Americans is higher than in Caucasians and Asians. Cytochrome P450 (CYP) 1B1 converts estrogens to the 4-hydroxy-catechol-estrogens. Studies show this catechol-estrogen to be mutagenic and may lead to prostate cancer. Polymorphisms of CYP1B1 have been associated with various types of cancers and recently, we have shown that CYP1B1 polymorphisms have higher risks for prostate cancer (Abstract; J. Urol. 171(Suppl. 4):111, 2004). However, such studies are lacking in race-related prostate cancer. There are at least 4 polymorphisms that have been identified on the CYP1B1 gene that results in a structural change in the enzyme and are at the following locations: codons 48 (C to G), 119 (G to T), 432 (C to G), and 453 (A to G). The main goal of this project is to investigate whether polymorphisms of the CYP1B1 gene can be a risk factor for race-related prostate cancer. To determine this, two specific aims are tested. In specific aim #1, the hypothesis that CYP1B1 gene is hyper-activated during malignant transformation of race-related prostate cells is tested. In the 2nd aim, the hypothesis that single nucleotide polymorphisms (SNPs) of the CYP1B1 gene have higher risk for race-related prostate cancer and correlate with hyper-activated CYP1B1 gene is tested. Data generated from these experiments will determine whether CYP1B1 gene expression differs between Caucasian and African-American prostate cancer samples. Also, these experiments will determine whether CYP1B1 SNPs are involved in race-related prostate cancer. This knowledge will help to understand the genetic basis for racial differences as well as identify the subjects who are at higher risk for prostate cancer.

BODY

Task #1. To determine if the CYP1B1 gene is differentially expressed between races (African-Americans and Whites) and in different stages and grades of prostate cancer.

Samples of benign prostatic hyperplasia (BPH) and prostate cancer are being collected. The methodology to determine CYP1B1 expression levels and activity are being developed and should be analyzed in the upcoming year.

Task #2. To determine if single nucleotide polymorphisms (SNPs) of the CYP1B1 gene are risk factors for the etiology of race-related prostate cancer and correlate with hyper-activity of its gene.

We have obtained samples of BPH (N=77) and prostate cancer (N=57) from African-American and Caucasian patients. DNA was collected from these samples by using a DNA extraction kit (Qiagen, Valencia, CA). Quantity and quality of DNA was measured at 260 nm and 280 nm by the use of a spectrophotometer. A two-step polymerase chain reaction (PCR) procedure was designed for the analysis of CYP1B1 polymorphisms. The primers of two of the polymorphic sites studied so far (codons 119 and 432) and PCR conditions are summarized in Table 1. In the first PCR, DNA (10 ng) was amplified in a 20 ul reaction containing 1.5 mM MgCl₂, 0.8 mM dNTP mix, PCR buffer, and 0.5 units of Red-Taq polymerase (Sigma-Aldrich, St. Louis, MO), along with primer sets designed to contain the polymorphic sites (Table 1). In the sequence-specific PCR (SSP), each polymorphic fragment was further amplified under similar conditions as the first-step PCR except for the use of SSP primer sets (Table 1). Each of the SSP products were electrophoretically separated on 3% agarose gels using 180 volts at ambient temperature. The products were then visualized by ethidium bromide staining under UV light. To confirm genotyping, products of the first PCR were subjected to direct DNA sequencing. DNA was purified from gels using a QIAquick PCR purification kit (Qiagen; Valencia, CA). Sequence analysis of purified products was then determined by using the first PCR primers and ABI 377 Sequencer and Dye Terminator Cycle sequencing kit (Applied Biosystems Inc.; Foster City, CA). Confirmation of DNA sequence was done on at least 3 representative samples for each of the polymorphic types. Frequencies of the various genotypes and allele types of CYP1B1 polymorphisms in the different categories of samples were determined and tabulated. Chi-square analysis was used to test each of the polymorphisms for differences in genotypic and allelic frequencies between

Whites and Blacks as well as between BPH and prostate cancer. Relative risk associated with a particular genotype or allele was estimated by calculating odds ratios (OR) along with 95% confidence intervals (CI).

Results to date of the genotypic and allelic frequencies of the two SNP sites of the CYP1B1 gene between African-Americans and Caucasians for both BPH and prostate cancer patients are shown in Tables 2 and 3, respectively. Interestingly among BPH patients, the variant genotype (G/G) and allele (G) at codon 432 are highly predominant in African-Americans as compared to Caucasians (Chi-square, $P < 0.01$). OR (95% CI) were 14.67 (4.20-51.24) for G/G genotype, and 3.76 (1.97-7.17) for the G allele in Blacks compared to Whites. However, no differences in genotype or allele were observed at codon 119 for BPH patients. In prostate cancer patients, although not significant, the codon 432 variant G/G is also much more prevalent in Blacks (52%) compared to Whites (28%). Non-significance may be due to the small N size. Allele frequency however, shows a difference as the codon 432 G allele displays an OR (95%CI) of 2.20 (1.05-4.61) in Blacks compared to Whites. No differences in genotype or allele frequency were detected at codon 119 between races among cancer patients. When comparing between BPH and prostate cancer patients within races, no differences were observed between the diseases amongst either African-Americans or Caucasians for both SNP sites studied.

We also analyzed the genetic distributions of six CYP1B1 SNPs among 200 Japanese, 200 Americans and 112 Germans by SSP. Frequencies of the variant at codon 119 and 432 were significantly lower in the Japanese population compared to that of the other populations. Only 5.5% of Japanese showed the 119 T/T variant compared to 9.0% Americans and 10.7% Germans. For codon 432, only 6.5 % Japanese displayed the G/G genotype compared to 14.5% Americans and 12.5% Germans. These data demonstrate that variants corresponding to hyper-activity of the CYP1B1 enzyme are different between races as they are low in frequency among Japanese.

Table 1. First step PCR and SSP primers utilized to determine SNP in BPH and prostate cancer samples

CODON 119

1st PCR

<u>Primer</u>	<u>Sequence</u>	<u>Anneal Temp</u>
C119-Rev	ccttccagtgtccgagtag	
C119-For	ccccatagtgggtgctgaatg	47 C

SSP

<u>Primer</u>	<u>Sequence</u>	<u>Anneal Temp</u>
C119 G-Rev with C119-For	acggaaggaggcgaaggc	65 C
C119 T-Rev with C119-For	acggaaggaggcgaagga	65 C

CODON 432

1ST PCR

<u>Primer</u>	<u>Sequence</u>	<u>Anneal Temp</u>
C432-Rev	tcatcactctgctggtcagg	
C432-For	gtcttgggtaccacattcc	47 C

SSP

<u>Primer</u>	<u>Sequence</u>	<u>Anneal Temp</u>
C432 C-Rev with C432-For	tccgggttaggccacttcag	65 C
C432 G-Rev with C432-For	tccgggttaggccacttcac	65 C

Table 2. Genotypic frequencies of CYP1B1 SNPs between races for BPH and Prostate cancer. P-value reflect chi-square test.

Type	Codon	Gene	White	Black	OR (95% CI)
BPH	119	G/G	26	20	Ref
		G/T	8	13	2.11 (0.76-5.89)
		T/T	5	5	1.30 (0.33-5.08)
	432	C/C	12	2	Ref
	P<0.01	C/G	18	14	4.67 (1.27-17.20)
		G/G	9	22	14.67 (4.20-51.24)
PC	119	G/G	20	12	Ref
		G/T	4	4	1.67 (0.35-7.94)
		T/T	6	8	2.22 (0.63-7.78)
	432	C/C	11	5	Ref
		C/G	12	7	1.28 (0.32-5.11)
		G/G	9	13	3.18 (0.89-11.35)

Table 3. Allele frequencies of CYP1B1 SNPs between races for BPH and Prostate cancer. P-value reflect chi-square test.

Type	Codon	Gene	White	Black	OR (95% CI)
BPH	119	G	60	53	Ref
		T	18	23	1.45 (0.71-2.95)
	432	C	42	18	Ref
	P<0.01	G	36	58	3.76 (1.97-7.17)
PC	119	G	44	28	Ref
		T	16	20	1.96 (0.88-4.38)
	432	C	34	17	Ref
	P=0.0644	G	30	33	2.20 (1.05-4.61)

KEY RESEARCH ACCOMPLISHMENTS:

- Obtained 77 BPH and 57 prostate cancer samples from African-American and Caucasian patients.
- Established primers and protocol to measure CYP1B1 SNPs.
- Evaluated CYP1B1 SNPs in BPH and prostate cancer samples.

REPORTABLE OUTCOMES:

The significances of the research performed to date are the following:

- 1) The CYP1B1 codon 119 T/T genotype has a higher risk for prostate cancer.
- 2) Differences in frequency of CYP1B1 SNPs occur between Americans, Japanese and Germans.
- 3) CYP1B1 codon 432 allele frequency differ between African-Americans and Caucasians with BPH or prostate cancer.

CONCLUSIONS:

Polymorphisms of the CYP1B1 gene are more frequent in patients with prostate cancer and therefore, can identify the population with higher risk for prostate cancer.

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Tanaka Y., M. Sasaki, H. Shiina, M. Igawa, M. Kaneuchi, C.J. Kane, P.R. Carroll, and R. Dahiya. 2004. Polymorphisms of Cytochrome P450 1B1 are risk factors for prostate cancer. J. Urol. 171(Suppl. 4):111, Abstract.

APPENDICES:

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